

# Effect of Potassium Lactate and a Potassium Lactate–Sodium Diacetate Blend on *Listeria monocytogenes* Growth in Modified Atmosphere Packaged Sliced Ham

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## ABSTRACT

Two commercially available organic acid salts, potassium lactate (PURASAL HiPure P) and a potassium lactate–sodium diacetate blend (PURASAL Opti.Form PD 4), were assessed as potential inhibitors of *Listeria monocytogenes* growth in modified atmosphere packaged (MAP) sliced ham in challenge studies. The influence of the initial inoculation level of *L. monocytogenes* ( $10^1$  or  $10^3$  CFU  $g^{-1}$ ) and storage temperature (4 or 8°C) was also examined. The addition of either organic acid salt to MAP sliced ham strongly inhibited the growth of *L. monocytogenes* during the normal shelf life of the product under ideal refrigeration conditions (4°C) and even under abusive temperature conditions (i.e., 8°C). During the challenge studies and in the absence of either organic acid salt, *L. monocytogenes* numbers increased by 1,000-fold after 20 days at 8°C and 10-fold after 42 days at 4°C. Both organic acid salt treatments were found to be listeristatic rather than listericidal. The addition of either organic acid salt to the MAP ham also reduced the growth of indigenous microflora, i.e., aerobic microflora and lactic acid bacteria. The influence of these compounds on the risk of listeriosis in relation to product shelf life is discussed.

Unlike most foodborne pathogens, *Listeria monocytogenes* can grow, albeit slowly, at refrigeration temperatures, in the presence of >5% salt, and in the absence of oxygen (37). Processed meats, including ham and pâté, are often packaged under vacuum or modified atmospheres and stored under refrigeration to extend their shelf life, typically being 6 to 8 weeks or more. Although initial contamination with *L. monocytogenes*, when present, is usually low, i.e., <10 CFU  $g^{-1}$  (15, 28), its potential for growth in ready-to-eat meats that are eaten without further cooking prior to consumption is, *prima facie*, considerable. Moreover, it is well documented that *L. monocytogenes* can grow in many such products during refrigerated storage, and such foods have caused, or been strongly implicated in, outbreaks of foodborne listeriosis that have resulted in fatalities (6–8, 23, 25).

*L. monocytogenes* present on raw ingredients can be killed reliably by the heat treatments routinely applied during smallgoods processing. During slicing and packaging after processing, however, recontamination can occur (16, 18, 24, 30, 39). During the past decade, the food industry has implemented hygiene practices and technologies that have greatly reduced the prevalence of *L. monocytogenes* on ready-to-eat foods (20, 28). Nevertheless, the ecology of the organism, particularly its ability to colonize food processing plants (38), still results in *L. monocytogenes* being detected, typically, in ~1 to 5%, or more, of processed meat products (1, 5, 15, 20, 28, 41).

One approach to minimize the risk of listeriosis is to

modify or treat long shelf life refrigerated products in such a way that the growth of *L. monocytogenes* is prevented, at least within the normal shelf life of the product. Organic acids and their salts, applied singly and in combination, have been shown by many studies to prevent or greatly retard the growth of *L. monocytogenes* in processed meats under both recommended temperatures of storage (at or below 4°C) and mild temperature abuse (e.g., up to 10°C). Generally, studies have focused on the activity of the sodium salts (2–4, 9, 14, 17, 18, 21, 22, 27, 29, 31, 34, 40), but several also consider potassium lactate or potassium lactate in combination with sodium diacetate (19, 26, 32, 33). Most studies have considered the activity of organic acid salts in sausage products (e.g., bratwurst, frankfurters, saveloys), but others have studied their effectiveness in cooked ham (4, 34). Mbandi and Shelef (21) considered the effectiveness in sterile uncooked comminuted beef emulsion.

There are a range of commercial products available based on the salts of organic acids that are intended to provide protection against the growth of undesirable microorganisms on foods. This project was undertaken to assess the antilisterial efficacy of two such products on an Australian smallgoods product, specifically, PURASAL HiPure P (potassium lactate) and PURASAL Opti.Form PD 4 (a potassium lactate–sodium diacetate blend). The influence, and possible interaction, of the presence of either antilisterial product, the initial level of *L. monocytogenes*, and the temperature of storage on the development of populations of *L. monocytogenes* in modified atmosphere packaged (MAP) sliced ham are investigated. The influence of indigenous microbiota is also considered.

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## MATERIALS AND METHODS

**Ham preparation.** Hams were prepared under commercial conditions by a large Australian manufacturer. During processing, antilisterial treatments were applied to hams prior to cooking. The treatments were as follows: the addition of 3% (wt/wt finished product) of PURASAL Opti.Form PD 4 (a blend consisting of 54.5 to 57.5% potassium lactate and 3.7 to 4.3% sodium diacetate; Purac Asia Pacific Pte. Ltd., Singapore) or the addition of 3% (wt/wt finished product) of PURASAL HiPure P (58 to 62% potassium lactate). Both products were provided to the smallgoods producer by Fibrisol Service Australia Pty. Ltd. (Heatherton, Victoria, Australia). Finished hams were sliced by the manufacturer and packaged in ~50-g lots into commercial thermoformed ridge bottom packs, composed of polyethylene terephthalate and polyethylene, and produced on horizontal form fill. The packages were then sealed under MAP (30% CO<sub>2</sub> and 70% N<sub>2</sub>). Control and treated samples were transported to the laboratory by commercial transport operators under refrigeration, but no temperature records were available.

**Bacterial strains.** Five strains of *L. monocytogenes* were used in combination for all "inoculated" samples. *L. monocytogenes* Scott A (type strain) and L5/22 (cold-smoked salmon isolate) were obtained from the School of Agricultural Science, University of Tasmania. Strains 20425, 20432, and 20423, all environmental isolates from a smallgoods factory, were supplied by Silliker Microtech Pty., Ltd., Melbourne, Victoria, Australia. Strains were maintained on bead suspensions in nutrient broth (Oxoid CM1, Oxoid Ltd., Hampshire, UK) with 15% glycerol and stored at -70°C.

**Preparation of inocula.** Before each experiment, isolates were resuscitated from cryogenic storage by plating onto brain heart infusion agar (Oxoid CM225) and incubating at 25°C for 48 h. A primary culture of each strain was prepared by touching a sterile loop to five colonies and inoculating 10 ml of prewarmed (25°C) tryptone soya broth (Oxoid CM129) containing 0.6% yeast extract (TSBYE; Oxoid L21). The primary culture for each strain was incubated without shaking at 37°C for 24 h. The primary cultures were then serially diluted in 0.1% peptone water (PW; Oxoid Bacteriological Peptone L37 containing 0.85% NaCl) and added to 50 ml of TSBYE broths in 125-ml side-arm flasks to achieve a level of approximately 10<sup>6</sup> CFU ml<sup>-1</sup>. The cultures were then incubated with shaking (80 oscillations min<sup>-1</sup>) in a water bath (Ratek Instruments Pty. Ltd., Victoria, Australia) at 10°C. Growth was monitored turbidimetrically (540 nm) until percent transmittance had decreased to ~20%. These steps were undertaken to generate exponentially growing inocula of ~10<sup>8</sup> CFU ml<sup>-1</sup> and acclimated to the chill temperatures considered representative of a smallgoods processing facility.

One-milliliter aliquots of the 10°C broth cultures of each of the five strains were added to a sterile 30-ml bottle and vortexed for 1 min. This "cocktail" suspension was then diluted in PW, prechilled to 10°C, to achieve levels of inocula such that final levels in inoculated samples were ~10<sup>1</sup> or ~10<sup>3</sup> *L. monocytogenes* CFU g<sup>-1</sup> ham. The amount of *L. monocytogenes* in the cocktail suspension was determined by viable count.

**Inoculation of ham.** Upon receipt at the University of Tasmania laboratories, control (no treatment) and treated samples were stored at 2°C for 2 days prior to inoculation with the *L. monocytogenes* cocktail and commencement of the challenge trial.

Low- and high-density inocula, prepared as described above, were aseptically injected onto the MAP sliced ham products (~50 g per package) with a hypodermic syringe to penetrate the pack-

aging. Uninoculated control samples were prepared by adding 0.1 ml of sterile PW. To minimize the potential for cross-contamination, these control samples were prepared prior to the samples requiring inoculation with the *L. monocytogenes*.

To preserve the integrity of the gas mixture, a self-adhesive rubber septum was applied to each sample package, and the syringe was inserted into the pack through the septum. An inoculum volume of 0.1 ml was added to a corner of the base of the package that did not contain any ham. The package was then inverted and gently shaken (by hand) so that the slices of ham moved across the inner surfaces of the container and each other. This was done to maximize the likelihood of even distribution of cells of *L. monocytogenes* or sterile diluent throughout the sample. Immediately after inoculation (or addition of sterile PW), the septum was covered with self-adhesive tape. Inoculated samples were appropriately labeled and incubated at either 4 or 8°C in walk-in cool-rooms. The duration of storage was ≤76 and ≤57 days for samples stored at 4 and 8°C, respectively.

**Sampling.** At appropriate time intervals, three samples from each treatment or control were assessed for levels of *L. monocytogenes*, concentrations of lactic acid bacteria, and aerobic plate count (APC). The entire sample from each package was aseptically removed and diluted 1:1 in PW and stomached for 2 min. Further 10-fold dilutions in PW were prepared as required, and 100- or 250-μl aliquots were surface plated with a spiral plater (Autoplate 4000, Spiral Biotech Inc., Bethesda, Md.) onto agar plates as follows. PALCAM plates (Oxoid CM877, and SR150 antibiotic supplement) for *L. monocytogenes* were incubated aerobically at 37°C for 48 h. The deMan Rogosa Sharpe agar (Oxoid CM361) plates for lactic acid bacteria were incubated aerobically at 25°C for 72 h. Plate count agar plates (Oxoid CM463 APHA Standard Plate Count Agar) for APCs were incubated at 20°C for 72 h. Colonies were counted manually, and log(viable cell count) of the triplicate samples was averaged (±standard deviation) and plotted against time. The maximum sample volume plated was 250 μl of the 1:1 dilution on quadruplicate plates. This permitted a maximum test sensitivity of 2 CFU g<sup>-1</sup>.

Water activity (Aqualab CX2, Decagon Devices, Pullman, Wash.) and direct measurement of the pH (pH meter 250A, Orion Research Inc., Boston, Mass.) of the ham were also determined from triplicate samples taken near the commencement of incubation and periodically throughout the trial.

## RESULTS

Because of the time taken to transport the hams from the commercial processor to the laboratory and to prepare the inoculum, the time between preparation of the hams and commencement of the challenge trial was 11 days. Changes in the microbial ecology and physicochemical attributes of the product were expected to commence from the time of packaging. Thus, all incubation times reported below relate to the time since commercial preparation, not the time of commencement of the challenge study. The manufacturer's shelf life recommendation for the untreated commercial product under the recommended refrigerated storage conditions was ~42 days.

The challenge trial was conducted once for each combination of control or antilisterial product, incubation temperature, and level of *L. monocytogenes*, with triplicate samples analyzed at each sampling time.

TABLE 1. pH changes over time in control and PURASAL-treated ham samples during storage at either 4 or 8°C (n = 3)<sup>a</sup>

Temp (°C)	Ham treatment	Sample time (days):						
		21	38	46	60	74	88	
4	Control (untreated, no <i>L. monocytogenes</i> )	6.44 ± 0.03	6.40 ± 0.13	NT	NT	6.15 ± 0.07	6.48 ± 0.06	
	Untreated, ~10 <sup>1</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.46 ± 0.02	6.44 ± 0.04	6.42 ± 0	6.19 ± 0.04	6.07 ± 0.03	6.43 ± 0.10	
	Untreated, ~10 <sup>3</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.40 ± 0.12	6.50 ± 0.03	6.44 ± 0.05	6.20 ± 0.05	6.10 ± 0.003	6.41 ± 0.11	
	Hi Pure P, ~10 <sup>1</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.40 ± 0.01	6.44 ± 0.02	6.48 ± 0.06	6.12 ± 0.05	6.19 ± 0.03	6.20 ± 0.04	
	Hi Pure P, ~10 <sup>3</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.48 ± 0.05	6.44 ± 0.03	6.47 ± 0.08	6.16 ± 0.03	6.23 ± 0.04	6.21 ± 0.03	
	Opti.Form PD 4, 10 <sup>1</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.47 ± 0.04	6.44 ± 0.03	6.45 ± 0.06	6.08 ± 0.10	6.24 ± 0.04	6.21 ± 0.03	
	Opti.Form PD 4, ~10 <sup>3</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.44 ± 0.05	6.45 ± 0.05	6.43 ± 0.04	6.19 ± 0.08	6.23 ± 0.04	6.22 ± 0.01	
Temp (°C)	Ham treatment	Sample time (days):						
		13	20	24	31	41	61	68
8	Control (untreated, no <i>L. monocytogenes</i> )	6.23 ± 0.05	6.14 ± 0.02	6.52 ± 0.06	6.20 ± 0.01	NT	5.85 ± 0.11	5.59 ± 0.24
	Untreated, ~10 <sup>1</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.21 ± 0.04	6.16 ± 0.04	6.45 ± 0.06	6.17 ± 0.04	6.37 ± 0.20	5.95 ± 0.10	5.71 ± 0.14
	Untreated, ~10 <sup>3</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.19 ± 0.02	6.18 ± 0.02	6.45 ± 0.03	6.20 ± 0.02	6.27 ± 0.21	5.84 ± 0.12	5.75 ± 0.17
	Hi Pure P, ~10 <sup>1</sup> CFU g <sup>-1</sup> <i>L. monocytognes</i>	6.24 ± 0.03	6.17 ± 0.01	6.45 ± 0.04	6.22 ± 0.01	6.49 ± 0.01	6.11 ± 0.02	6.22 ± 0.03
	Hi Pure P, ~10 <sup>3</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.23 ± 0.04	6.16 ± 0.04	6.46 ± 0.03	6.14 ± 0.02	6.44 ± 0.01	6.23 ± 0.09	6.17 ± 0.03
	Opti.Form PD 4, ~10 <sup>1</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.21 ± 0.05	6.18 ± 0.03	6.45 ± 0.03	6.19 ± 0.02	6.53 ± 0.10	6.15 ± 0.07	6.20 ± 0.03
	Opti.Form PD 4, ~10 <sup>3</sup> CFU g <sup>-1</sup> <i>L. monocytogenes</i>	6.25 ± 0.06	6.19 ± 0.03	6.43 ± 0.02	6.16 ± 0.04	6.46 ± 0.06	6.22 ± 0.08	6.16 ± 0.03

<sup>a</sup> NT, not tested.

**Water activity, gas mixture, and pH changes.** The water activity of control and treated ham samples showed little change during storage at either 4 or 8°C (data not shown). Moreover, the addition of either a sterile diluent or small volume of inoculum to the hams did not affect water activity. Overall, untreated hams had a higher average water activity ( $0.969 \pm 0.003$ ;  $n = 27$ ) than hams treated with either the potassium lactate–sodium diacetate blend ( $0.959 \pm 0.005$ ;  $n = 18$ ) or potassium lactate ( $0.959 \pm 0.002$ ;  $n = 18$ ).

The pH values of the control (untreated) and treated ham samples over time of incubation at 4 and 8°C are shown in Table 1. The pattern of response was initially similar for all ham types at 4°C. The pH remained constant at ~6.4 up to 46 days postprocessing and then declined to ~6.2. Thereafter, the pH of treated hams remained unchanged. Untreated hams, both inoculated and uninoculated, returned to a pH similar to that at commencement of the trial. At 8°C, pH changes were similar for all hams up to 31 days postprocessing, with pH fluctuating between ~6.2 and 6.4. This pattern continued for the duration of the trial for hams treated with either antilisterial agent. For

untreated hams, both uninoculated and inoculated with *L. monocytogenes*, the pH declined after 40 days to ~5.6 to 5.7.

The gas composition of selected samples, assessed periodically throughout the trial, showed no systematic changes (data not shown).

**Untreated control ham.** The data for the uninoculated and inoculated untreated hams provide the baseline against which the efficacy of the two treatments can be assessed. The APC reached maximum population density by day 54 at 4°C and day 34 at 8°C for both uninoculated and untreated hams inoculated with either low or high numbers of *L. monocytogenes* (Figs. 1a through 1c and 2a through 2c). In all cases, the APC mainly consisted of lactic acid bacteria. *L. monocytogenes* was not recovered from uninoculated control samples stored at either 4 or 8°C (Figs. 1a and 2a) with a level of detection of <2 CFU g<sup>-1</sup>.

*L. monocytogenes* inoculated into untreated samples and stored at 4 or 8°C showed growth. At 4°C, approximately 1 log of growth was observed 42 days after inoculation for ham with either low (Fig. 1b) or high (Fig. 1c)

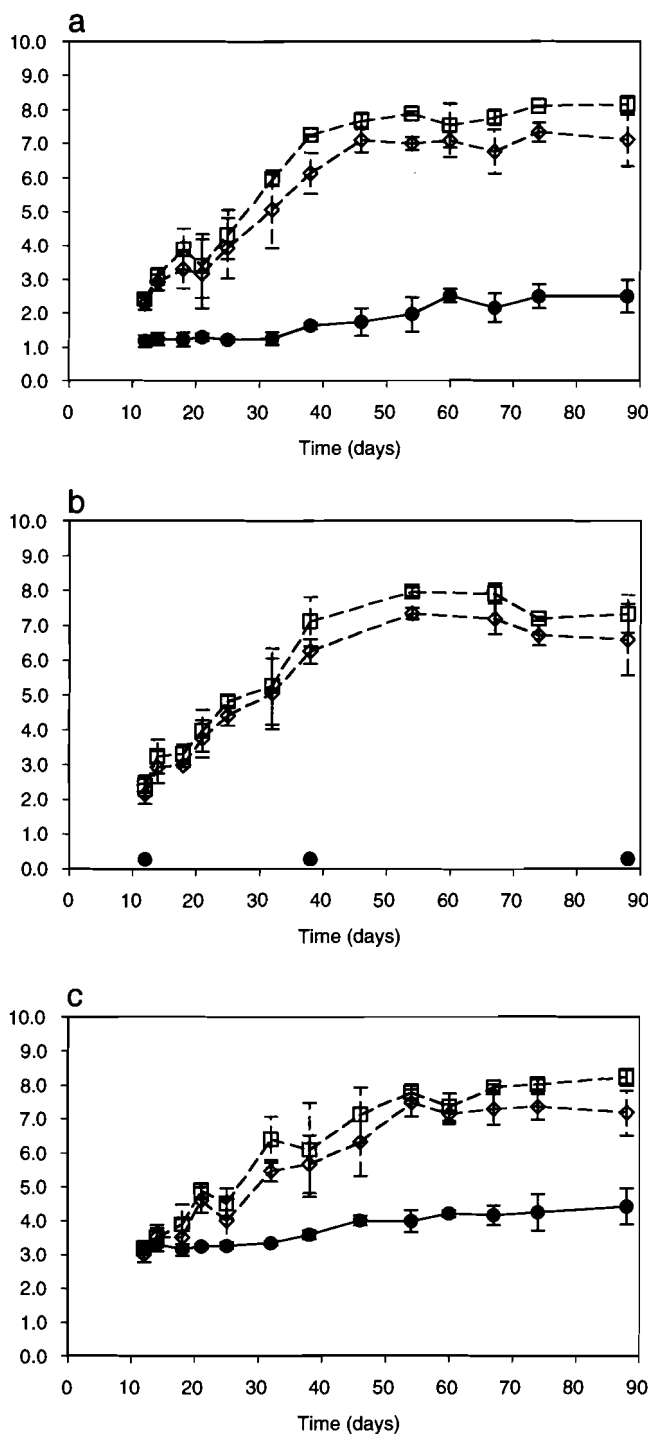


FIGURE 1. Growth of aerobic microflora (—□—), lactic acid bacteria (—◇—), and *L. monocytogenes* (●) in untreated modified atmosphere packaged sliced ham stored at 4°C where (a) uninoculated control, (b) inoculated with low numbers ( $\sim 10^1$  CFU  $g^{-1}$ ) of *L. monocytogenes*, and (c) inoculated with high numbers ( $\sim 10^3$  CFU  $g^{-1}$ ) of *L. monocytogenes*. Standard deviation ( $n = 3$ ) is also plotted. For uninoculated control samples, data plotted represent a determination of  $<2$  CFU  $g^{-1}$ .

added numbers of *L. monocytogenes*. More growth was observed,  $\sim 3$  log, at 8°C after 20 days (postinoculation) and was also independent of inoculum size (Fig. 2b and 2c). Initiation of growth at 4°C appeared to be considerably delayed,  $\sim 20$  days postinoculation, compared with that at

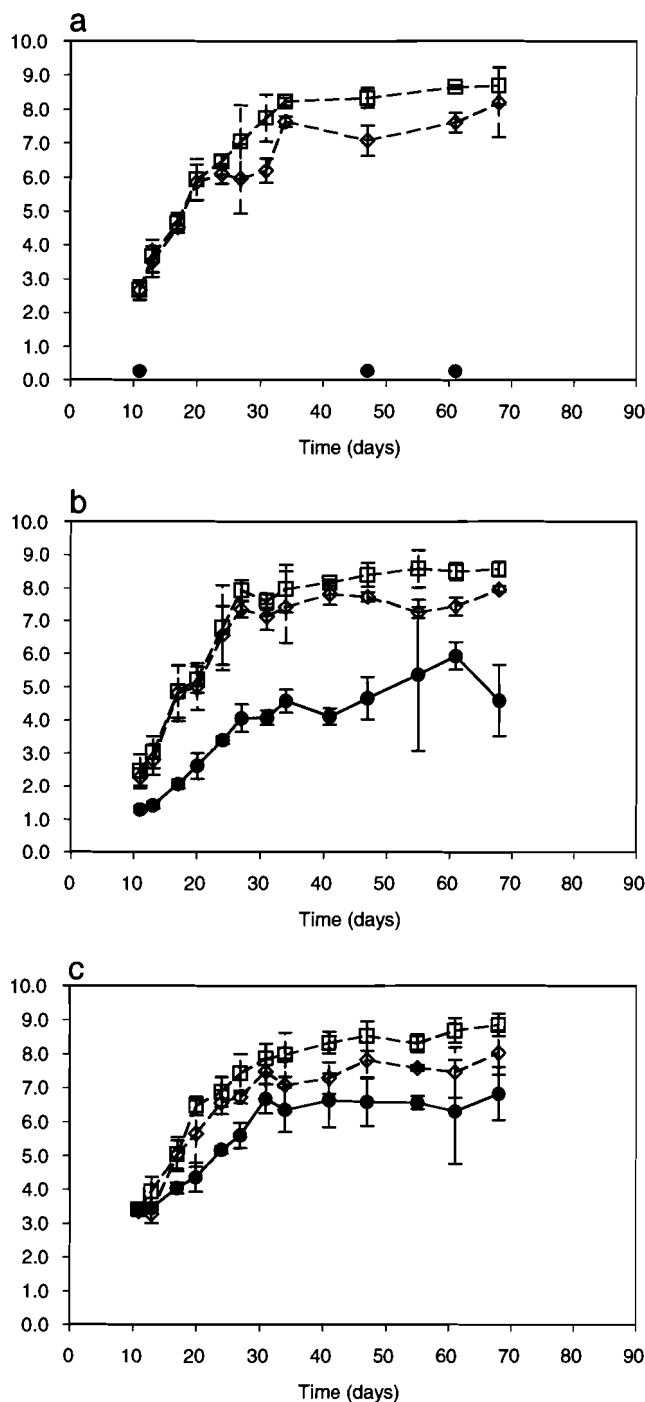


FIGURE 2. Growth of aerobic microflora (—□—), lactic acid bacteria (—◇—), and *L. monocytogenes* (●) in untreated modified atmosphere packaged sliced ham stored at 8°C where (a) uninoculated control, (b) inoculated with low numbers ( $\sim 10^1$  CFU  $g^{-1}$ ) of *L. monocytogenes*, and (c) inoculated with high numbers ( $\sim 10^3$  CFU  $g^{-1}$ ) of *L. monocytogenes*. Standard deviation ( $n = 3$ ) is also plotted. For uninoculated control samples, data plotted represent a determination of  $<2$  CFU  $g^{-1}$ .

8°C,  $\sim 2$  days. In all inoculated and untreated hams, the growth of *L. monocytogenes* in the product appeared to plateau when the total number of viable aerobic bacterial cells in the samples reached  $\sim 10^8$  CFU  $g^{-1}$ .

**Treated ham.** Figures 3a and 3b and 4a and 4b show the effect of the addition of the potassium lactate-sodium

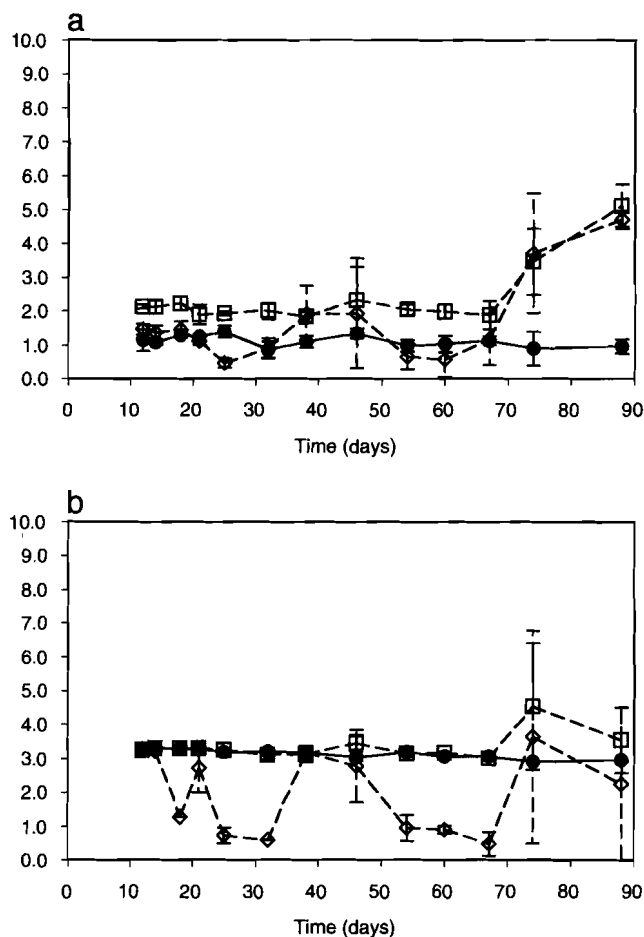


FIGURE 3. Growth of aerobic microflora (—□—), lactic acid bacteria (—◇—), and *L. monocytogenes* (●) at 4°C in modified atmosphere packaged sliced ham treated with potassium lactate-sodium diacetate (PURASAL Opti.Form PD 4) and inoculated with either (a) low numbers ( $\sim 10^1$  CFU g<sup>-1</sup>) or (b) high numbers ( $\sim 10^3$  CFU g<sup>-1</sup>) of *L. monocytogenes*. Standard deviation ( $n = 3$ ) is also plotted.

diacetate blend, and Figures 5a and 5b and 6a and 6b show the effect of potassium lactate on the changes in microbial populations, including added *L. monocytogenes*, in MAP sliced ham. In all cases, it was apparent that the treatments greatly reduced the extent of microbial growth when compared with untreated hams (Figs. 1b and 1c and 2b and 2c).

Regardless of inoculum level, the growth of *L. monocytogenes* was prevented for 76 days (postinoculation) at 4°C in both potassium lactate-sodium diacetate (Fig. 3a and 3b) and potassium lactate (Fig. 5a and 5b) treated hams. Similarly, no growth of *L. monocytogenes* was observed on potassium lactate-treated hams after 57 days (postinoculation) at 8°C (Fig. 6a and 6b). A small increase ( $\sim 0.5$  log CFU g<sup>-1</sup>) in *L. monocytogenes* numbers was apparent in the last samples taken, i.e., 57 days postinoculation, for potassium lactate-sodium diacetate treated hams stored at 8°C. With the Student's *t* test, the difference between the mean of those counts and the mean of the preceding counts was found to be significant ( $P < 0.05$ ) for the samples with low *L. monocytogenes* inoculum (Fig. 4a) and highly significant ( $P < 0.001$ ) for the samples with high *L. monocytogenes* inoculum (Fig. 4b).

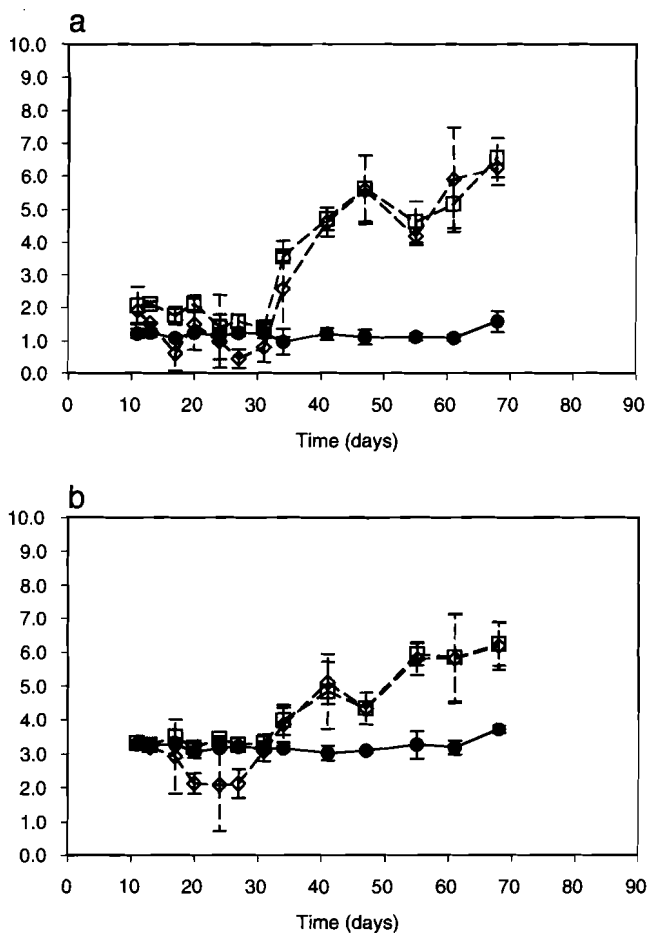


FIGURE 4. Growth of aerobic microflora (—□—), lactic acid bacteria (—◇—), and *L. monocytogenes* (●) at 8°C in modified atmosphere packaged sliced ham treated with potassium lactate-sodium diacetate (PURASAL Opti.Form PD 4) and inoculated with either (a) low numbers ( $\sim 10^1$  CFU g<sup>-1</sup>) or (b) high numbers ( $\sim 10^3$  CFU g<sup>-1</sup>) of *L. monocytogenes*. Standard deviation ( $n = 3$ ) is also plotted.

Both treatments at both temperatures also suppressed the growth of indigenous bacteria in terms of growth rate and time of initiation of growth compared with untreated hams. It is important to note that the APC includes the introduced *L. monocytogenes* so that, in some cases, the growth of indigenous bacteria that are present at concentrations less than  $\sim 10^3$  CFU g<sup>-1</sup> can be masked by the nongrowing *L. monocytogenes* population in the high inoculum treatments.

For hams treated with potassium lactate-sodium diacetate, the APC remained unchanged for 55 days (postinoculation) at 4°C, regardless of *L. monocytogenes* inoculum level (Fig. 3a and 3b). Thereafter, numbers increased slowly, in comparison to control hams, to  $\sim 5$  and  $4.5$  log CFU g<sup>-1</sup> in low and high inoculated hams, respectively. At 8°C, APC growth was suppressed for 20 days postinoculation. Thereafter, the APC increased to  $\sim 6$  log CFU g<sup>-1</sup> at a slower rate than observed in control hams inoculated with *L. monocytogenes* (Fig. 2b and 2c).

For hams treated with potassium lactate, the APC remained relatively unchanged during storage at 4°C, i.e., for 76 days postinoculation (Fig. 5a and 5b). A small increase

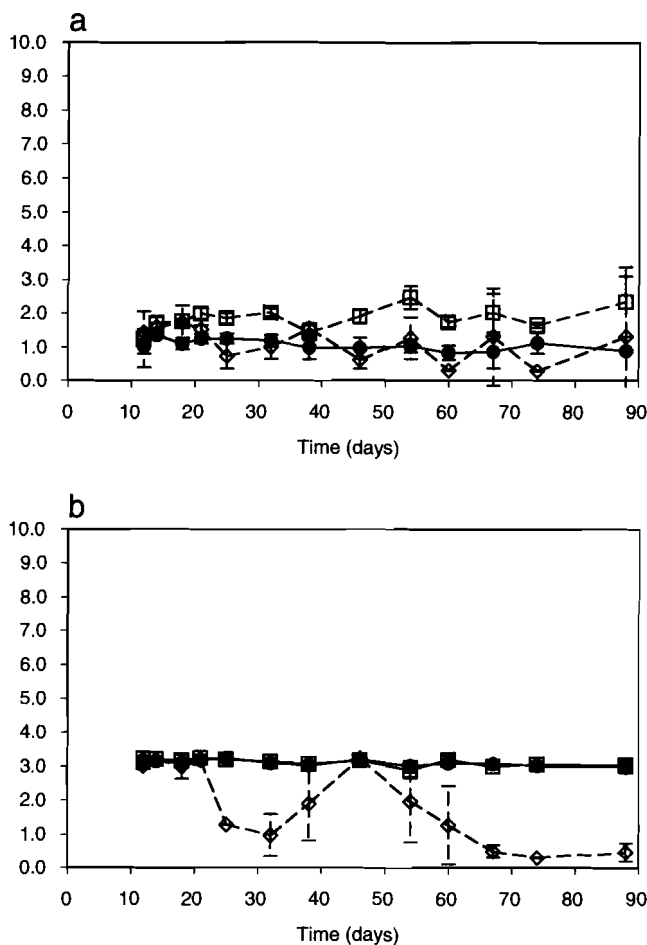


FIGURE 5. Growth of aerobic microflora (—□—), lactic acid bacteria (—◇—), and *L. monocytogenes* (●) at 4°C in modified atmosphere packaged sliced ham treated with potassium lactate (PURASAL HiPure P) and inoculated with either (a) low numbers ( $\sim 10^1$  CFU  $g^{-1}$ ) or (b) high numbers ( $\sim 10^3$  CFU  $g^{-1}$ ) of *L. monocytogenes*. Standard deviation ( $n = 3$ ) is also plotted.

in numbers may have occurred in the hams inoculated with low numbers of *L. monocytogenes* (Fig. 5a); however, the response fluctuated over the duration of storage. Note that the APC mainly consists of *L. monocytogenes* in the high inoculated hams (Fig. 5b), thus masking the response of indigenous microflora. As for potassium lactate–sodium diacetate treated hams at 8°C (Fig. 4a and 4b), the APC is suppressed for the first 20 days of storage postinoculation. Thereafter, the APC increases. If the APC data for all treated and inoculated hams are plotted together, it is apparent that the rate of increase is slower in potassium lactate–treated hams than in those treated with the potassium lactate–sodium diacetate blend (data not shown).

The response of indigenous lactic acid bacteria in the treated and inoculated hams was similar to that of the APC, particularly when growth was observed. However, when the APC remained static, it was apparent that the numbers recovered on deMan Rogosa Sharpe varied between sampling times. For example see Figure 3b for hams treated with potassium lactate–sodium diacetate, inoculated with high numbers of *L. monocytogenes*, and stored at 4°C.

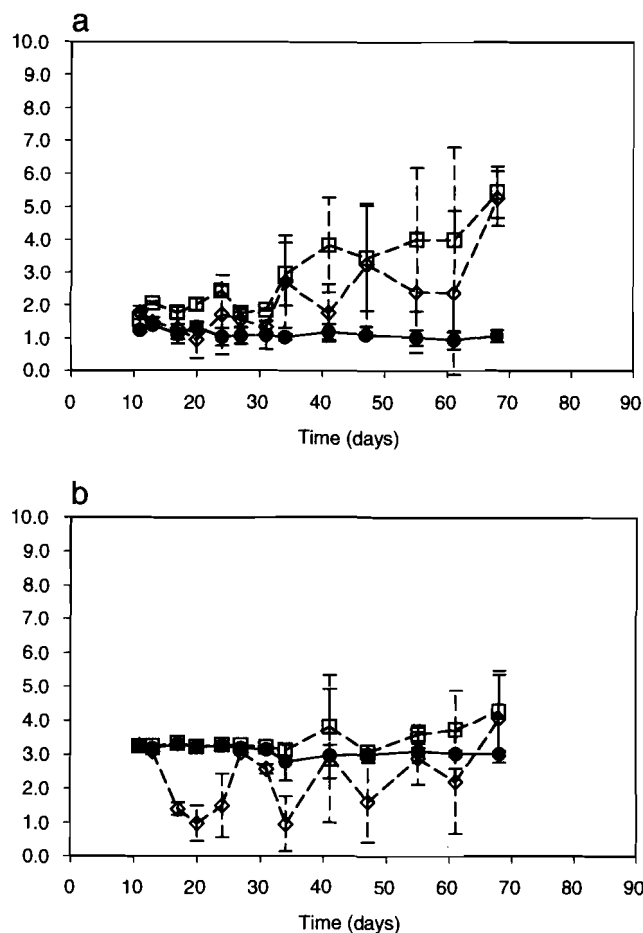


FIGURE 6. Growth of aerobic microflora (—□—), lactic acid bacteria (—◇—), and *L. monocytogenes* (●) at 8°C in modified atmosphere packaged sliced ham treated with potassium lactate (PURASAL HiPure P) and inoculated with either (a) low numbers ( $\sim 10^1$  CFU  $g^{-1}$ ) or (b) high numbers ( $\sim 10^3$  CFU  $g^{-1}$ ) of *L. monocytogenes*. Standard deviation ( $n = 3$ ) is also plotted.

## DISCUSSION

The five *L. monocytogenes* strains used in these experiments were grown to late exponential phase and acclimated to growth at chill temperature (10°C) to minimize the lag time on introduction to the ham environment. This approach appears to have been successful as, in the untreated and inoculated controls (Fig. 2b and 2c) at 8°C, there is little evidence of a lag phase before the commencement of growth. At 4°C, however, there is a lag time of  $\sim 20$  days (Fig. 1a and 1b). The use of a mixture of five strains of *L. monocytogenes* was intended to minimize the possibility that any observed inhibition of growth observed would be strain-specific.

To more clearly illustrate the effects of the two treatments on the potential for *L. monocytogenes* growth in MAP stored ham, Figure 7a and 7b presents a direct comparison of the population growth of *L. monocytogenes* in untreated controls, and the respective treatments, at each of the storage temperatures and *L. monocytogenes* inoculum levels studied. The results support the reported effectiveness of both potassium lactate and combined potassium lactate and sodium diacetate under both recommended storage

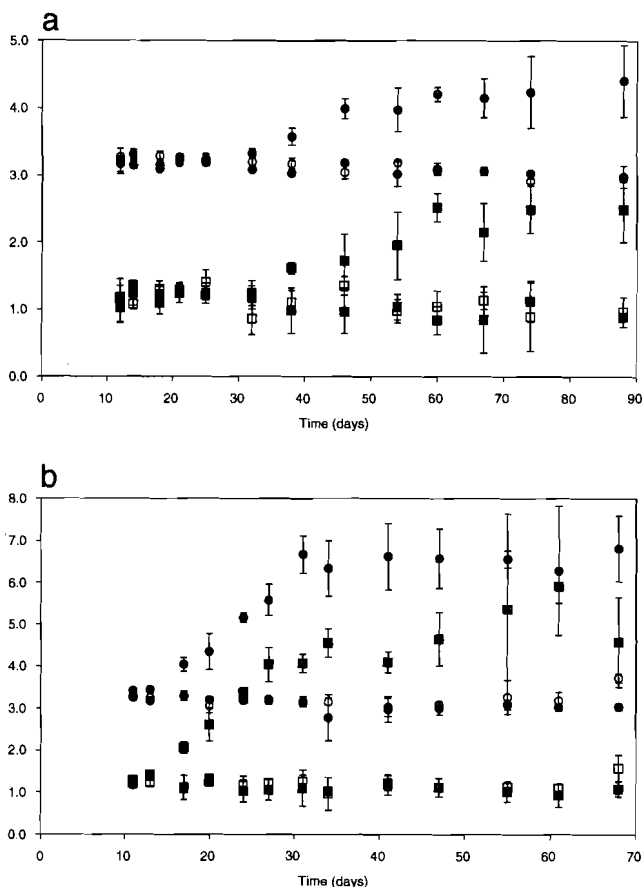


FIGURE 7. Growth at (a) 4°C and (b) 8°C of either high (circles) or low (squares) levels of *L. monocytogenes* inoculated into ham treated with either potassium lactate–sodium diacetate (PURASAL P Opti.Form PD 4; open symbols) or potassium lactate (PURASAL HiPure P; closed symbols) compared with untreated control samples (shaded symbols). Standard deviation ( $n = 3$ ) is also plotted.

conditions (4°C) and inappropriate storage conditions (8°C) for MAP ham. Although growth was clearly possible in the untreated ham product, the addition of the potassium lactate–sodium diacetate treatment almost completely eliminated the growth of *L. monocytogenes* at 4 or 8°C (Figs. 3a and 3b and 4a and 4b), while hams treated with potassium lactate completely suppressed the growth of *L. monocytogenes* at 4°C for 90 days (Fig. 5a and 5b) and for 60 days at 8°C (Fig. 6a and 6b). Consistent with other reports (3, 4, 14, 18, 21, 22, 26, 29, 32–34), neither treatment was listericidal at the levels used but was listeristatic. Samelis et al. (29), however, found that 6% lactate was listericidal in some processed meat products.

An additional level of protection against the growth of *L. monocytogenes* in MAP or vacuum-packed products may be afforded by the presence and growth of lactic acid bacteria. Lactic acid bacteria have an ecological advantage in such products and usually become numerically dominant during storage (36) but without necessarily causing spoilage of the product. Homofermentative lactic acid bacteria, in particular, can reach high concentrations in vacuum-packed or MAP products but without causing overt spoilage (12). The presence of high concentrations of other bacteria cre-

ates an additional hurdle to the growth of organisms present in lower numbers. This phenomenon has been termed the Jameson Effect (10, 11, 13, 35). Expressed simply, the Jameson Effect describes the often-observed phenomenon that when one microbial species in an environment reaches stationary phase, all other species present also enter stationary phase, irrespective of whether they are at levels normally associated with their maximum population density. The phenomenon is not yet fully explained but can account for the observations summarized in Figure 7a and 7b in which the maximum population density achieved by introduced *L. monocytogenes* appears to be dependent on their initial density in the challenge trial. We interpret this as occurring because the concentration of other bacteria initially present on the ham was the same, irrespective of the level of introduced *L. monocytogenes*, and because those bacteria reached stationary phase at the same time during the respective challenge trial conditions (i.e., 4 or 8°C). The growth of *L. monocytogenes* was suppressed after the same amount of time. Consequently, the final levels reached by *L. monocytogenes* in the product are dependent on the levels initially introduced. Figure 2b and 2c illustrates the effect most clearly by showing that the cessation of the growth of *L. monocytogenes* corresponds closely with the onset of stationary phase of the aerobic microflora, which is dominated by the lactic acid bacteria. This effect was also noted by Stekelenburg (33). Glass et al. (14) also commented on the contribution of lactic acid bacteria to suppression of pathogen growth in long shelf life processed meats and stated that “manufacturers must optimise the critical balance between increasing shelf life and permitting the growth of spoilage lactic acid bacteria that compete with *L. monocytogenes* for nutrients.”

Although full characterization of the likely reduction in risk of listeriosis due to use of listeristatic agents such as organic acid salts would require extensive modeling, general trends can be inferred from the results in this study. Given the low number of *L. monocytogenes* typically present on processed meats at the point of manufacture, the delay in growth induced by the presence of organic acid salts (and CO<sub>2</sub>), in combination with the growth-suppressing effects of high concentrations of lactic acid bacteria or other indigenous bacteria, would be expected to reduce the risk of listeriosis from vacuum-packed or MAP processed meats. This is expected because, once any *L. monocytogenes* present was able to grow, other bacteria might have reached concentrations high enough to suppress *L. monocytogenes* growth, i.e., by the Jameson Effect. The magnitude of this suppression might also depend on the relative concentrations of lactic acid bacteria and *L. monocytogenes* initially present. For this reason, it is important to undertake studies with realistic initial levels of *L. monocytogenes* (e.g.,  $\leq 10$  CFU g<sup>-1</sup>), as noted by Porto et al. (26) and the present study.

The beneficial effect of the addition of organic acid salts in reducing risk from *L. monocytogenes* could be lessened, however, if the growth suppression applied equally to indigenous bacteria and product shelf lives were extended as a result. Inhibition of the growth of lactic acid bacteria

and APC was seen in this study (Figs. 3 through 6) and potentially enables an extension of product shelf life. Samelis et al. (29) noted less pH reduction during prolonged storage of untreated controls than with organic acid salt treated pork frankfurters. Similar results were observed in this study for ham stored at 8°C (Table 1). This is possibly because of the inhibition of lactic acid bacteria growth and delay of spoilage processes. Stekelenburg (33) reported inhibition of the growth of lactic acid bacteria and resultant extension of shelf life of 75 to 125%, and in an earlier study, Stekelenburg and Kant-Muermans (34) reported that the growth of *L. monocytogenes* was more inhibited than the growth of a lactic acid bacterium, *Lactobacillus curvatus*. Extension of shelf life to exploit the inhibition of growth of other microorganisms could lessen the antilisterial benefits of organic acid salt treatments, because the growth of *L. monocytogenes*, while delayed by the treatment, might eventually be possible because of the extended shelf life of the product. Shelf lives of 75 to 90 days for some processed meats, including frankfurters, are expected in United States (29). Shelf lives in Europe are, apparently, similar to those in Australia (28). Stekelenburg and Kant-Muermans (34) indicate that shelf lives of 3 to 4 weeks at 7°C are common. We concur with Glass et al. (14) that manufacturers and retailers need to remain mindful of their reasons for using organic acid additives to processed meats and that the interplay between *L. monocytogenes* risk minimization and product shelf life extension is clearly understood. Optimization of *L. monocytogenes* risk reduction compared with shelf life extension achieved by application of salts of organic acids may need to be evaluated on a case-by-case basis.

Bedie et al. (3) considered that “injured cells may be overlooked or underestimated, despite their potential to repair damage, and proliferate in foods to potentially become a risk.” This factor should be considered in the interpretation of the results presented here for the recovery of *L. monocytogenes* on PALCAM agar. Results presented in Figures 4 through 7, however, suggest that PALCAM is a reliable enumeration medium. During the study, we observed that both *L. monocytogenes* and lactic acid bacteria were recovered on deMan Rogosa Sharpe agar (data not shown). From those same figures, whereas the numbers of *L. monocytogenes* estimated by their growth on PALCAM were constant, the numbers of colonies recovered on deMan Rogosa Sharpe agar often fell below the level recovered on PALCAM (e.g., Fig. 5b). The reasons for this phenomenon are unknown and were not explored further in this study.

In general, the results of this study are consistent with previously published reports concerning cured meat products and demonstrate that organic acid salts are powerful listeristatic agents. The addition of organic acid salts to cured meats could reduce the risk of listeriosis from processed meats by preventing the growth of *L. monocytogenes*, usually initially present at low levels only, to levels less likely to cause human illness. The microbial ecology of refrigerated vacuum-packed or MAP processed meats is complex, particularly the interplay between product formulation and growth potential of lactic acid bacteria and

spoilage bacteria and pathogenic contaminants. This suggests that the potential for shelf life extension achievable with additions of organic acid salts must be considered together with the potential for *L. monocytogenes* contamination and growth in the product. Further studies to characterize these interactions are being undertaken.

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